Piezo-ICSI

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Piezo-ICSI

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Learning Objectives

- To review the current techniques for intracytoplasmic sperm injection (ICSI) in human oocytes
- To clarify the problems in the current ICSI technique (Conventional-ICSI)
- To introduce a new technology of ICSI (Piezo-ICSI) to improve the survival and fertilization rates for injected oocytes
- To survey how Piezo-ICSI can contribute to the human assisted reproductive technology field

Key Points

- In the Conventional-ICSI technique, the cytoplasm is aspirated into the micropipette to break the membrane.
- The volume of cytoplasm aspirated into the micropipette at the membrane breakage point affects the fertilization rate after ICSI.
- In the Piezo-ICSI technique, the cytoplasm is not aspirated into the micropipette.
- Piezo-ICSI results in higher survival and fertilization rates than Conventional-ICSI.
- The micropipette wall thickness used for Piezo-ICSI affects the survival and fertilization rates after ICSI.
- Piezo-ICSI can contribute to shortening the training period for ICSI for junior embryologists.

39.1 Introduction

39.1.1 Brief History of Intracytoplasmic Sperm Injection (ICSI)

The first four pregnancies achieved by ICSI were reported by Palermo in 1992 [1], and ICSI is now an essential technique in human assisted reproductive technology (ART). ICSI uses beveled and spiked micropipettes (Fig. 39.1a) for mechanical penetration of the zona pellucida and the membrane as well as aspiration of the cytoplasm into the micropipette to break the membrane. After membrane breakage, the sperm is injected into the cytoplasm (Conventional-ICSI). However, the survival rate of mouse oocytes (oocyte diameter 80 μm) was as low as 16% (8% fertilization rate) after Conventional-ICSI [2].

Kimura and Yanagimachi performed membrane breakage by applying a piezo pulse, which produced ultrafast submicron forward momentum using uniquely shaped flat-tipped micropipettes with no bevel or spike (Fig. 39.1b) (Piezo-ICSI), in 1995 for mouse oocytes [2]. The survival rate of mouse oocytes was dramatically improved to 80% (78% fertilization rate) by using Piezo-ICSI [2]. Therefore, the Piezo-ICSI may be a less invasive method also for human oocytes (oocyte diameter 160 μm). However, to the best of our knowledge, only four reports detail the application of Piezo-ICSI to human oocytes, and little information is available regarding its clinical efficiency [3–6].

The goal of this chapter is to compare Conventional-ICSI and Piezo-ICSI techniques and to show the superiority of the Piezo-ICSI technique.

39.1.1.1 Conventional-ICSI Method

We used commercially available ICSI micropipettes with a beveled and spiked tip (Fig. 39.1a) (K-MPIP-1035, Cook Ireland Ltd., Ireland). The micropipette inner diameter was 5 μm, and the wall thickness was 1 μm. The micropipette was connected to a pneumatic injector (IM-9C, NARISHIGE Inc., Japan). The micropipette preparation was as follows. First, HEPES-buffered medium (SYDNEY IVF GAMETE BUFFER, Cook Australia Pty Ltd., Australia) was aspirated into the micropipette by capillary action for 1 min. Next, 7% polyvinylpyrrolidone (PVP) (7% PVP solution, Irvine Scientific, USA) was aspirated via negative pressure using an air injector. A motile sperm was immobilized by crushing the tail with the micropipette tip and aspirated tail-first into the micropipette in a 10 μl drop of 7% PVP. With the polar body at 12 o'clock, the micropipette was inserted through the zona pellucida into the oocyte (~90% of the oocyte diameter) to stretch the membrane (Fig. 39.2a–c). The membrane breakage procedure was performed as follows. Air was aspirated into the micropipette using an air injector to create negative pressure and suction on the membrane. The membrane was slowly aspirated into the micropipette (Fig. 39.2d) until a sudden flow of cytoplasm into the micropipette occurred (Fig. 39.2e), which was considered to be the moment of membrane breakage. After membrane breakage, positive air pressure was quickly provided to stop the flow of cytoplasm into the micropipette, and the sperm was injected into the oocyte (Fig. 39.2f).

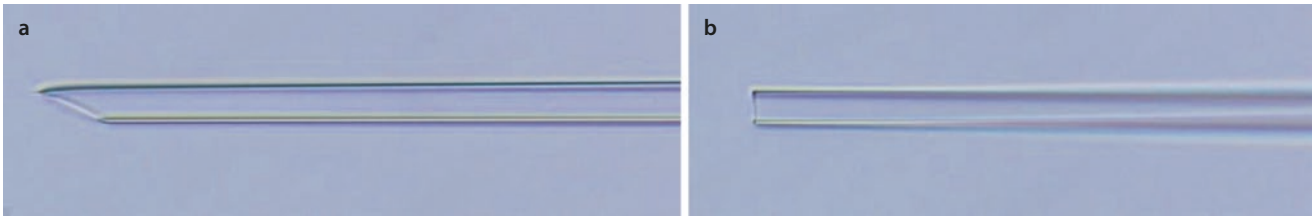

Fig. 39.1 Micropipettes for Conventional-ICSI **a** and Piezo-ICSI **b**

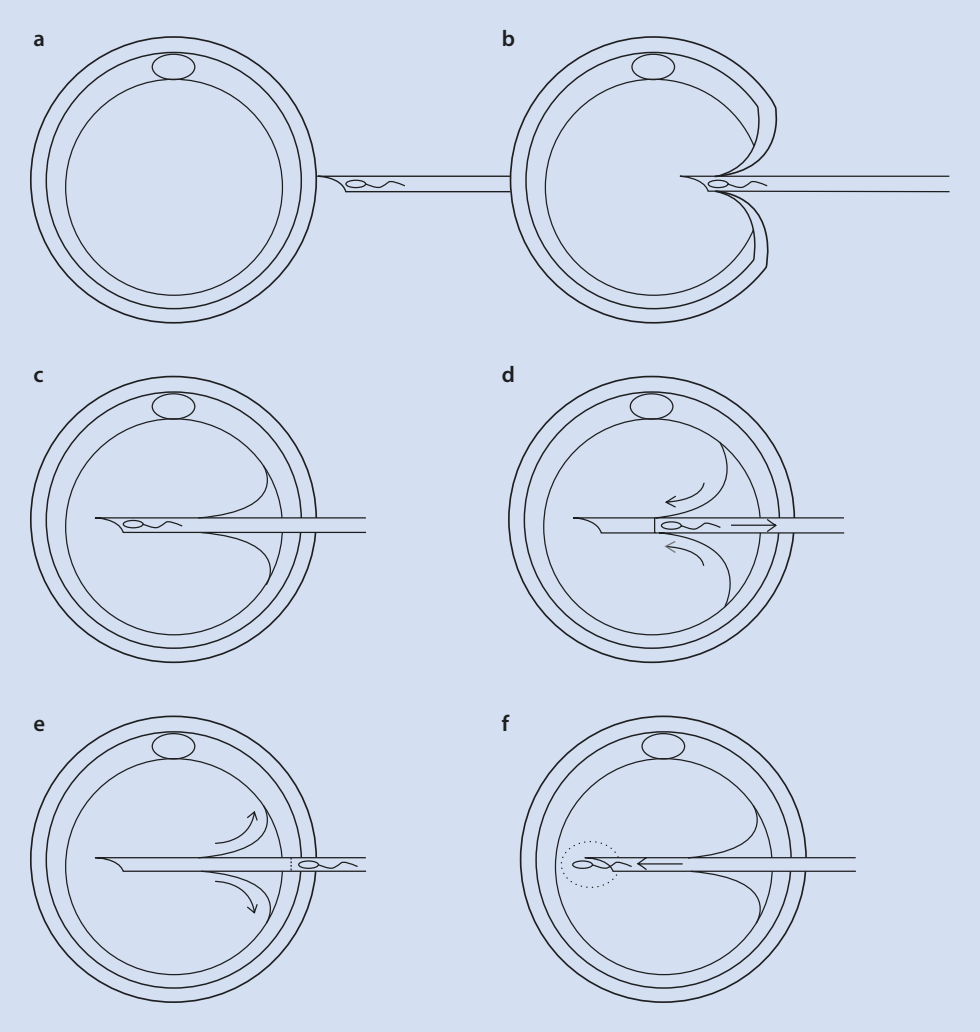

Fig. 39.2 Conventional-ICSI Before zona drilling **a**, during zona drilling **b**, after zona drilling **c**, during cytoplasm aspirating into the micropipette **d**, membrane breakage **e,** and sperm injection **f**

39.1.1.2 Piezo-ICSI Method

Characteristics of Piezo-ICSI

In Piezo-ICSI, membrane breakage is performed by applying a piezo pulse that produces ultrafast submicron forward momentum using uniquely shaped flat-tipped micropipettes with no bevel or spike (Piezo-ICSI) (Fig. 39.1b) [2]. During zona penetration, the injection pipette can penetrate the zona without zona or oocyte deformation, and during membrane breakage, no cytoplasm is aspirated into the micropipette.

Procedure for Piezo-ICSI

We used commercially available Piezo-ICSI micropipettes with a flat tip (PIN07-20FT, PRIME TECH Ltd., Japan). Fluorinert (6.25 µl, FC-770, 3 M) was aspirated to the middle of the micropipette (Fig. 39.3). Fluorinert is a clear, colorless, fully fluorinated liquid, which is nontoxic and water insoluble. The micropipette was inserted and clamped into the micropipette holder, which was then connected to the oil injector (HDJ-M3, PRIME TECH Ltd.). The piezo-micromanipulator drive unit (MB-S, PRIME TECH Ltd.) was attached to the micropipette holder

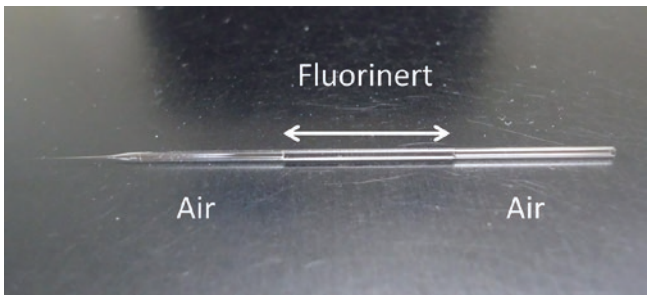

Fig. 39.3 Fluorinert placed in the middle of the micropipette for Piezo-ICSI

(Fig. 39.4). The piezo drive unit was driven by a controller (PMAS-ET150, PRIME TECH Ltd.). After Fluorinert was pushed to the micropipette tip, 6–12 pl of 7% PVP was aspirated into the micropipette. The sperm was then immobilized, as was done for Conventional-ICSI, and aspirated tail-first into the micropipette. Without oocyte deformation, the micropipette was placed gently against the zona pellucida while piezo pulses were applied to allow the pipette to break through the zona pellucida and not the membrane (Fig. 39.5a–c). The broken piece of the zona was expelled, and the sperm was advanced

Fig. 39.4 Piezo-micromanipulator drive unit for Piezo-ICSI

until the sperm head was near the micropipette tip (Fig. 39.5d). The micropipette was advanced forward (to ~90% of the oocyte diameter) to stretch the membrane (Fig. 39.5e, f). The membrane break was performed by applying one piezo pulse without aspirating the cytoplasm into the micropipette (Fig. 39.5g), and the sperm was injected into the oocyte (Fig. 39.5h).

Mechanism of Piezo-ICSI

Figure 39.6 shows the mechanism of zona pellucida opening or membrane breakage by piezo pulse. The Fluorinert is placed in the middle of the micropipette (Fig. 39.6a). The piezo pulse is applied producing an ultrafast submicron

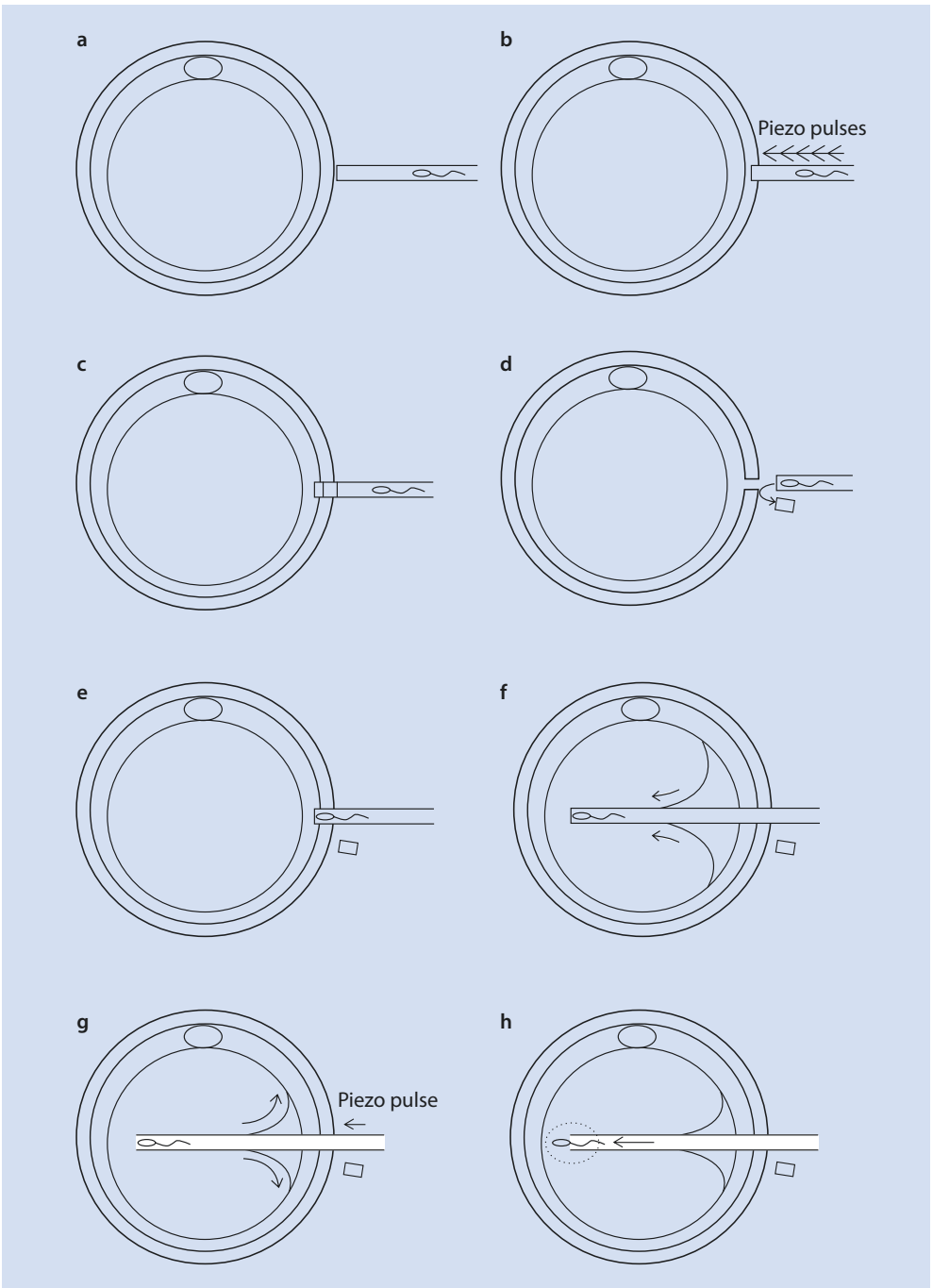

Fig. 39.5 Piezo-ICSI Before zona drilling **a**, during zona drilling **b**, after zona drilling **c**, expelling the broken piece of the zona **d**, during insertion of the micropipette tip through the drilled hole **e**, during stretching the membrane **f**, membrane breakage by applying the piezo pulse **g,** and sperm injection **h**

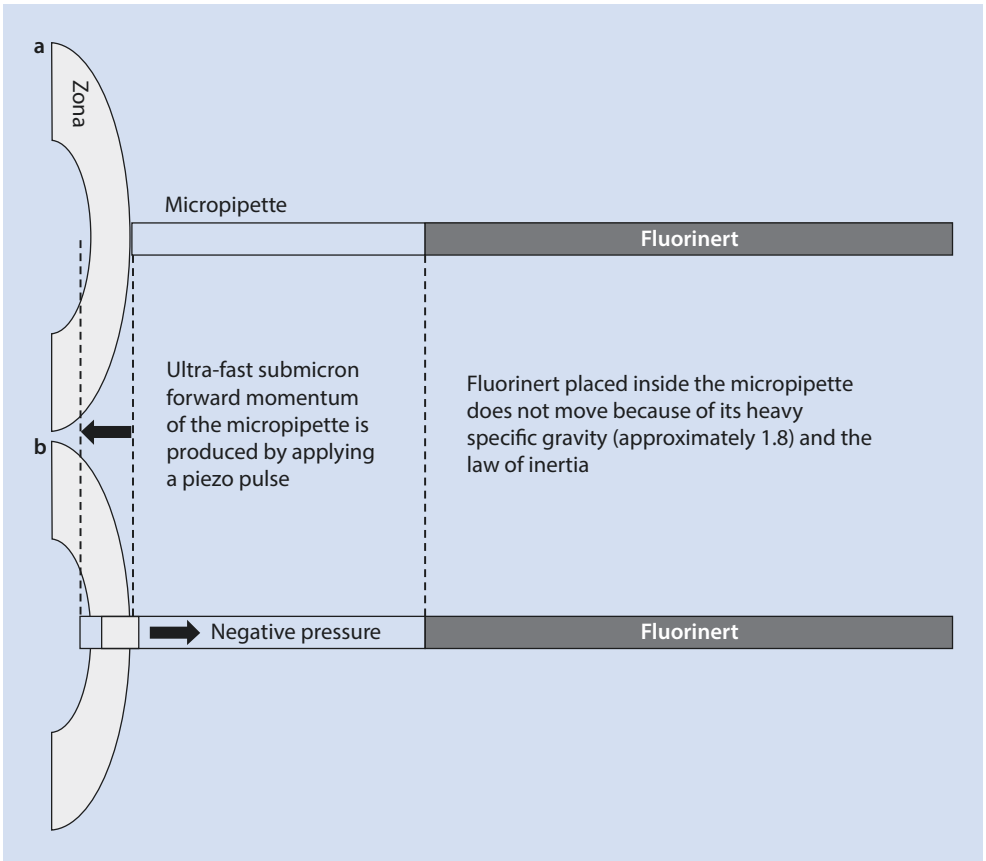

Fig. 39.6 Mechanism of Piezo-ICSI Before applying a piezo pulse **a** and during applying a piezo pulse **b**

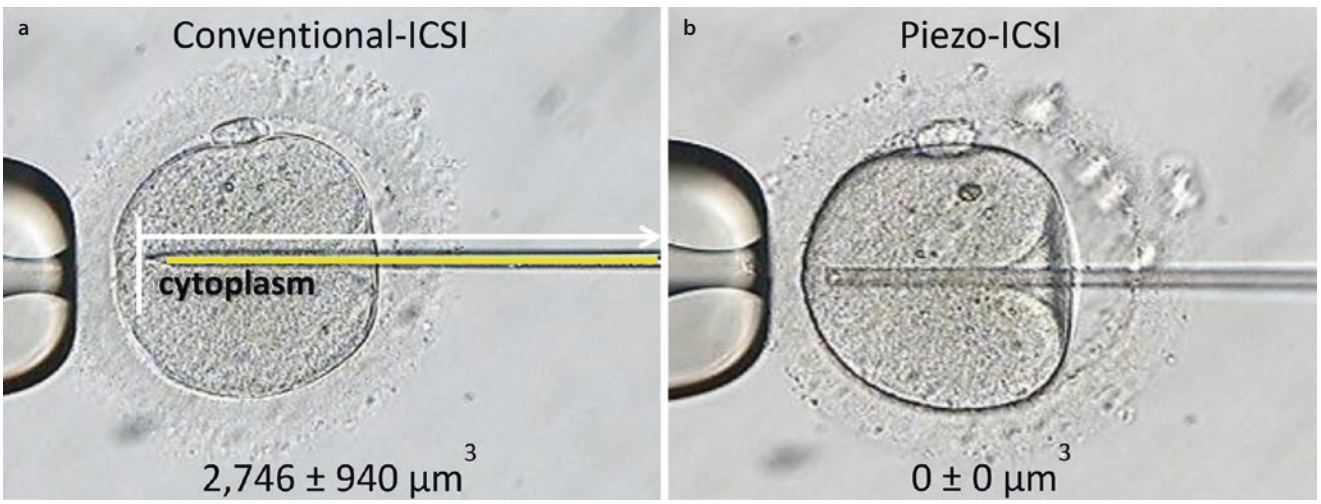

Fig. 39.7 Mean volume of cytoplasm aspirated into the micropipette at the point of membrane breakage from Conventional-ICSI **a** and Piezo-ICSI **b**

forward momentum of the micropipette. By applying a piezo pulse, only the micropipette is advanced forward, whereas the Fluorinert inside the micropipette does not move because of its heavy specific gravity (approximately 1.8) and the law of inertia. As a result, ultrafast submicron negative pressure is produced inside the micropipette tip. This ultrafast submicron negative pressure produces ultrafast submicron aspiration at the micropipette tip. This ultrafast aspiration induces the opening of the zona pellucida or membrane breakage (Fig. 39.6b). However, if the power of the piezo pulse is stronger, the zona or membrane can be destroyed, so the mechanism of Piezo-ICSI is still unclear.

39.1.1.3 Comparison Between Conventional-ICSI and Piezo-ICSI

In our previous analysis of 1341 oocytes from 286 patients, the calculated mean volume of cytoplasm aspirated into the micropipette with Conventional-ICSI (2746 ± 940 µm^3) (Fig. 39.7a)

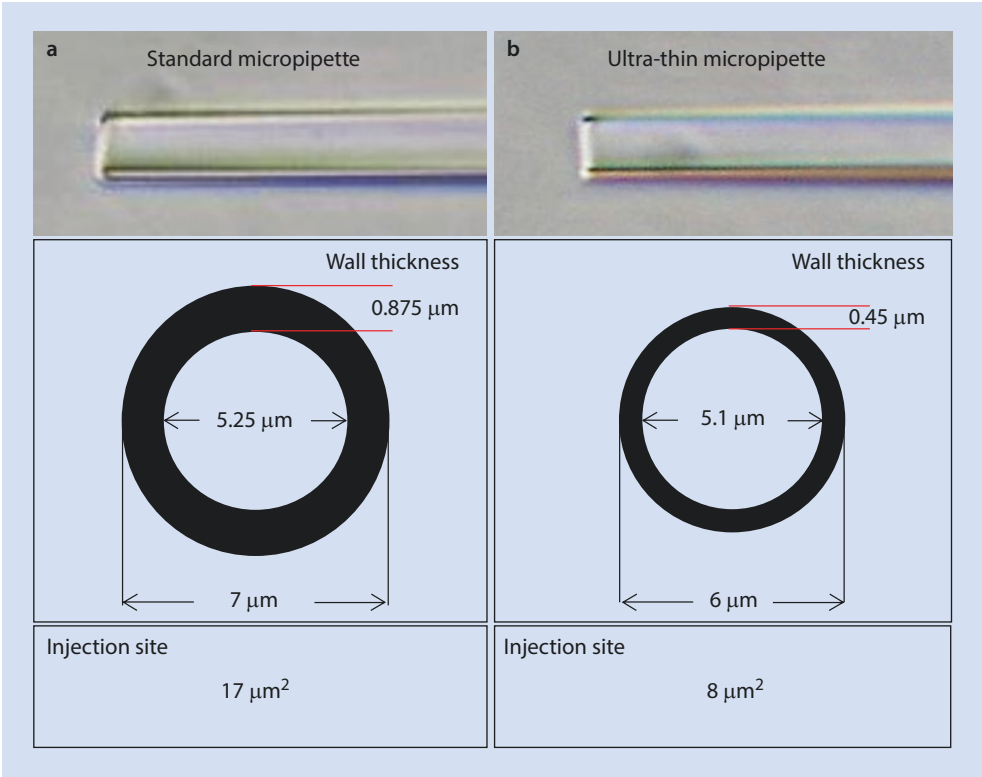

Fig. 39.8 Wall thickness, inside diameter, and injection site in the membrane for a standard micropipette **a** and an ultrathin micropipette **b**

was significantly higher than with Piezo-ICSI (0 ± 0 μm^3) (Fig. 39.7b) ($P < 0.05$) [6]. In addition, significantly higher survival and fertilization rates were observed when using Piezo-ICSI (717 oocytes from 166 patients) compared to Conventional-ICSI (624 oocytes from 120 patients) (survival rates 95% vs. 90%, fertilization rates 75% vs. 68%) ($P < 0.05$) [6]. When using the Conventional-ICSI method, the injection site in the membrane was larger due to the procedure of aspirating the cytoplasm into the micropipette during membrane breakage, which is avoided when using Piezo-ICSI. Moreover, Conventional-ICSI might also increase physical damage to the oocyte. As a result, the survival and fertilization rates using Conventional-ICSI were significantly lower than when using Piezo-ICSI. However, no significant differences were observed in embryo quality and pregnancy, implantation, or live birth rates between Conventional-ICSI and Piezo-ICSI. These results suggest that Piezo-ICSI can increase survival and fertilization rates without detrimental effects on embryo quality, implantation ability, or live birth potential.

39.1.1.4 Improvement in the Piezo-ICSI Technique

Standard Micropipette Versus Ultrathin Micropipette

We evaluated two micropipette wall thicknesses to determine the effect on the rates of survival, fertilization, good-quality day 3 embryos, pregnancy, implantation, and live birth during Piezo-ICSI. The standard micropipette had a wall thickness of 0.875 μm (Fig. 39.8a), and the ultrathin micropipette had a wall thickness of 0.45 μm (Fig. 39.8b). The membrane injection sites for the standard micropipette and ultrathin micropipette were 17 μm^2 and 8 μm^2, respectively (Fig. 39.8a, b) [6]. In our previous analysis of 1396 oocytes from 317 patients, significantly higher rates for survival (99% vs. 95%), fertilization (89% vs. 75%), good-quality day 3 embryos (55% vs. 43%), pregnancy (31% vs. 21%), implantation (31% vs.21%), and live births (25% vs.16%) were obtained when using the ultrathin micropipette (679 oocytes from 151 patients) than when using the standard micropipette (717 oocytes from 166 patients) for Piezo-ICSI ($P < 0.05$) [6]. The physical damage to the oocyte was reduced by creating a smaller injection site (17 μm^2 vs. 8 μm^2), which could partially explain the increased survival and fertilization rates. Consequently, we suggest that the combination of Piezo-ICSI and the ultrathin micropipette can significantly improve the effective utilization rate of injected oocytes and can increase live birth rates.

Oil Injector Versus Air Injector

In preparation for Piezo-ICSI, we aspirated approximately 1–2 cm of Fluorinert to the middle of the micropipette (Fig. 39.3). Next, this micropipette was inserted into the micropipette holder of the oil injector filled with mineral oil. The mineral oil flows inside the micropipette, pushing the air and Fluorinert forward to the micropipette tip. Figure 39.9a shows a good example of micropipette preparation. However, if air bubbles occur in the mineral oil (Fig. 39.9b) while inserting the micropipette into the micropipette holder, Piezo-ICSI does not work. A hole does not open in the zona, and the membrane does not break. In this case, this micropipette is discarded. Because

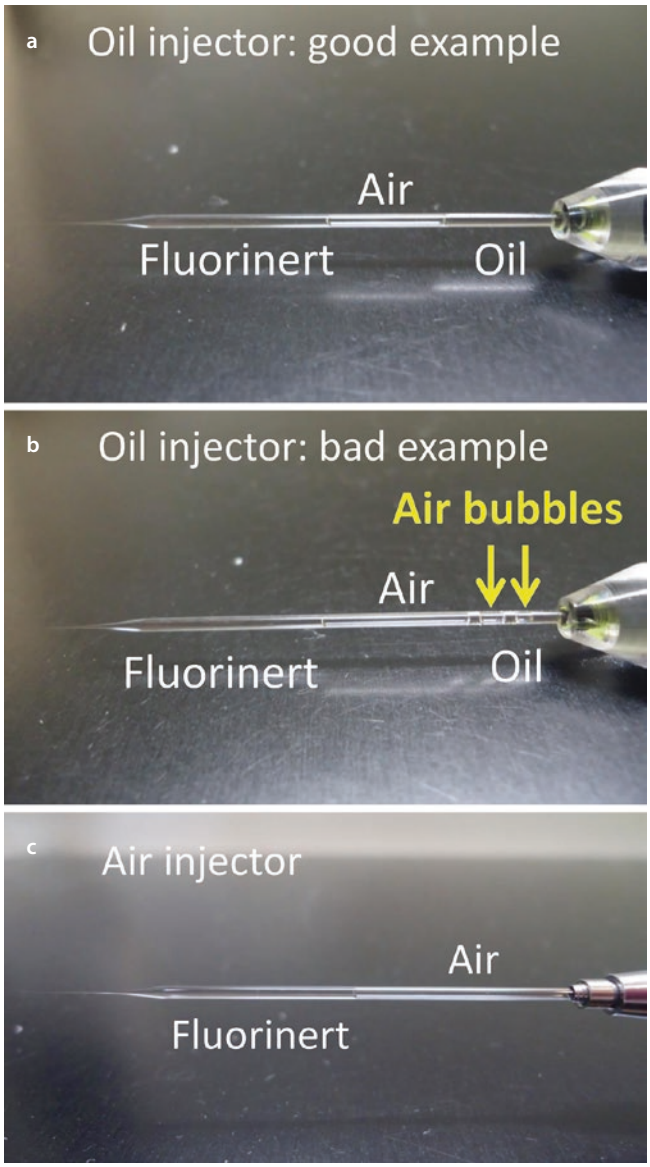

Fig. 39.9 Preparation of Piezo-ICSI using an oil injector (**a**: good example, **b**: bad example) and air injector **c**

the oil injector is sticky from the mineral oil, micropipette preparation takes time. Therefore, if an air injector for Piezo-ICSI can be used, the number of wasted micropipettes and preparation time should be reduced. However, little information is available regarding the clinical efficacy of Piezo-ICSI with an air injector. Therefore, we assessed the clinical efficiency and safety of Piezo-ICSI with an air injector. In our previous analysis of 780 oocytes from 180 patients, we measured the time for micropipette preparation with the oil injector (409 oocytes from 90 patients) and air injector (371 oocytes from 90 patients). The average time for the oil injector was 233 s, whereas the average time was significantly reduced to 106 s with the air injector ($P < 0.05$) [7]. We also counted the number of wasted micropipettes due to air bubbles. The average number of wasted micropipettes from the oil injector per patient was 0.28 ± 0.56. However, when using the air injector, which was free from mineral oil and thus air bubbles were not possible (Fig. 39.9c), no micropipettes were wasted [7]. No significant differences were found between the oil and air injectors in the survival (99% vs. 99%), fertilization (89% vs. 90%), or good-quality day 3 embryo (61% vs. 61%) rates [7]. During this study, an extra 3.2 h and 25 micropipettes were used for the oil injector group (90 patients) compared to the air injector group (90 patients) [7]. Our results indicate that the air injector dramatically reduced the waste of time and micropipettes occurring with the oil injector and did not impair the survival, fertilization, or good-quality day 3 embryo rates. Therefore, Piezo-ICSI with an air injector is clinically efficient and safe.

Head-First Injection Versus Tail-First Injection

In human oocyte ICSI, a sperm is injected head-first into the cytoplasm during fertilization because sperm internalization into the cytoplasm is initiated from the sperm head in natural fertilization. However, ICSI procedures bypass hyperactivation, zona pellucida penetration, and internalization of the sperm head into the cytoplasm. Because the sperms are injected directly into the cytoplasm, the oocytes could be fertilized if injected tail-first. However, little information is available regarding the effect of the sperm direction during injection into the cytoplasm for Piezo-ICSI results and embryo development. In order to inject sperm head-first into the cytoplasm, the sperm is aspirated from the tail into the micropipette. This procedure is technically difficult owing to the small tail size. Head-first sperm aspiration into the micropipette would be easier and faster. Therefore, we assessed the effects of sperm direction (head-first or tail-first) during injection into the cytoplasm on oocyte survival, fertilization, and embryo development. For head-first injection, the sperm was aspirated into the micropipette tail-first and injected into the oocyte head-first (Fig. 39.10a); for tail-first injection, the sperm was aspirated into the micropipette head-first and injected into the oocyte tail-first (Fig. 39.10b). In our previous analysis of 632 oocytes from 152 patients, we calculated the duration of sperm manipulation (from starting sperm immobilization to aspiration of the sperm into the micropipette). The average time for sperm manipulation during head-first injections (342 oocytes from 75 patients) was 10.5 ± 1.6 s, whereas with the tail-first injections (290 oocytes from 77 patients), the time was significantly reduced to 8.6 ± 1.8 s ($P < 0.05$) [8]. No significant difference was found between the head- and tail-first injections in the survival rates (99% vs. 99%), fertilization rates (86% vs. 90%), or good-quality day 3 embryo rates (69% vs. 68%) [8]. Our results indicate that the sperm direction during cytoplasmic injection does not affect oocyte survival, fertilization, and subsequent embryo development (good-quality day 3 embryo rate) with Piezo-ICSI. However, aspiration of sperm into the micropipette is easier and faster using sperm tail-first instead of head-first. Consequently, we recommend injecting the sperm into the cytoplasm tail-first during Piezo-ICSI.

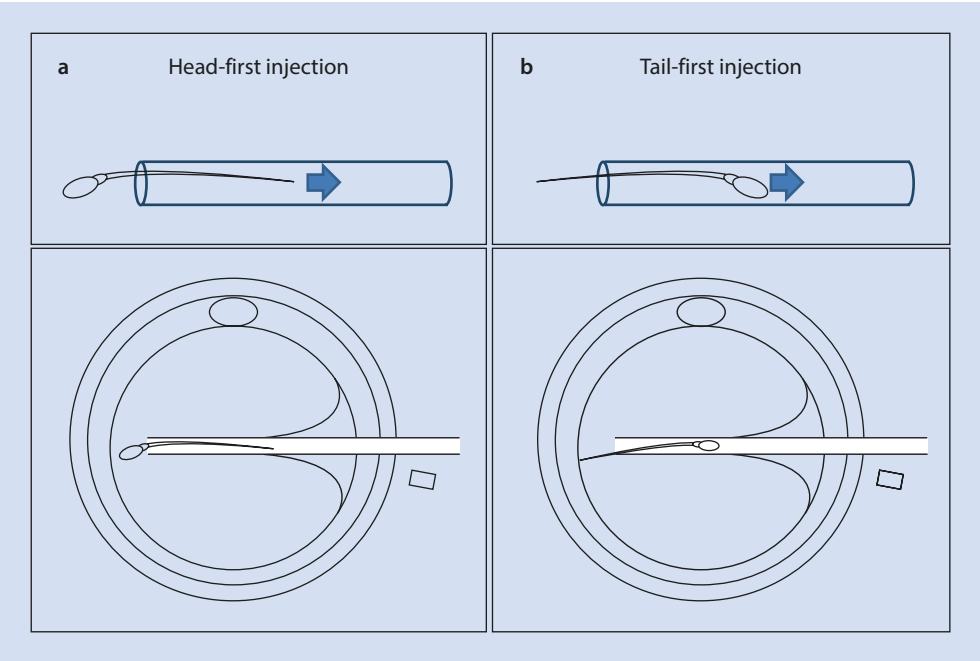

Fig. 39.10 Head-first injection **a** and tail-first injection **b** during Piezo-ICSI

39.1.1.5 Advantages of Piezo-ICSI

Improvement in Fertilization Rate and Reduced ICSI Training Period

The fertilization rates for Conventional-ICSI published in the 2000s were 62–77% [9–13]. Similarly, the fertilization rate for Conventional-ICSI performed by three junior embryologists at our hospital, Kameda Medical Center (Kamogawa City, Chiba, Japan), was 66%. We sought to improve this fertilization rate using Piezo-ICSI. However, little information was available regarding if Piezo-ICSI improves the fertilization rate for procedures performed by junior embryologists and, if so, how many procedures are needed to improve the rate. We assessed whether introduction of Piezo-ICSI could improve the fertilization rates and, if so, the number of procedures needed to see improvement. The study subjects were three junior embryologists. They had performed Conventional-ICSI for 5, 5, and 1 year, respectively. They received Piezo-ICSI training from a senior embryologist who had performed Conventional-ICSI for 11 years and Piezo-ICSI for 4 years. The fertilization rate for the procedures performed by the senior embryologist at Kameda Medical Center was 83%. The fertilization rate for the senior embryologist per 20 oocytes (120 oocytes in total) was more than 80%. Thus, we considered our junior embryologists proficient in performing Piezo-ICSI when their fertilization rate improved to ≥80% per 20 oocytes. In our previous analysis of 1373 oocytes, the fertilization rate for Conventional-ICSI (between February 2014 and September 2014) performed by the three junior embryologists was 66%, whereas the fertilization rate with Piezo-ICSI (between October 2014 and June 2015) significantly improved to 82% ($P < 0.05$) (Fig. 39.11). The fertilization rates for Conventional-ICSI performed by junior embryologists I, II, and III were 60%, 74%, and 64%, respectively. The fertilization rates for Piezo-ICSI performed by junior embryologists I, II, and III were 80%, 83%, and 83%, respectively. The fertilization rates in the case of each junior embryologist using Piezo-ICSI were significantly higher than that for Conventional-ICSI ($P < 0.05$). After 20 procedures, the fertilization rates from the junior embryologists using Piezo-ICSI reached ≥80% per 20 oocytes (unpublished data). Our results indicate that Piezo-ICSI significantly improved the fertilization rates for the procedures performed by three junior embryologists from 66% to 82%, and they became proficient after 20 procedures.

39.2 Summary

The rates of survival and fertilization using Piezo-ICSI with standard micropipettes in our previous results (survival rate 95%, fertilization rate 75%) are not superior to those recently reported using Conventional-ICSI (survival rate 89–93%, fertilization rate 62–77%) [9–13], suggesting that Piezo-ICSI with a standard micropipette may not be optimized for human oocytes. However, Piezo-ICSI with ultrathin micropipettes resulted in significantly higher survival and fertilization rates than with standard micropipettes (survival rates 99% vs. 95%, fertilization rates 89% vs. 75%) [6]. Furthermore, as shown in this chapter, we designed a new Piezo-ICSI methodology using an air injector and tail-first injection for a more user-friendly Piezo-ICSI technique. By using this modified Piezo-ICSI, we significantly improved the ICSI fertilization rate performed by our junior embryologists from 66% to 82% after 20 procedures. We believe that Piezo-ICSI can not only improve the ICSI fertilization rate but also shorten the training period for ICSI practitioners. Further study is needed to assess whether Piezo-ICSI can improve the fertilization rate and shorten the training period for ICSI at other ART institutions.

Fig. 39.11 Monthly and total fertilization rates of Conventional-ICSI (between February 2014 and September 2014) and Piezo-ICSI (between October 2014 and June 2015) for three junior embryologists at Kameda Medical Center

Review Questions

1. What kind of characteristic does the current ICSI technique (Conventional-ICSI) have during membrane breakage?
2. What kind of characteristic does a new technology of ICSI (Piezo-ICSI) have during membrane breakage?
3. How can Piezo-ICSI contribute to the training period for ICSI for junior embryologists?

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